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Research paper

Eucalyptus x urograndis biomass production for energy purposes exposed to a Mediterranean climate under different irrigation and fertilisation regimes

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ABSTRACT

Lignocellulosic biomass derived from energy crops, a renewable energy source, must be boosted in order to mitigate climate change effects. For this reason, vegetative growth and biomass production of *Eucalyptus x urograndis*, under a Mediterranean climate, was studied for three years. At the second and the third planting years, 12 treatments were applied combining four irrigation levels during the dry season (0, 325, 646 and 1298 mm of water per year, plus 418 mm of average rainfall) and three fertilisation amounts (0, 150 and 300 kg ha^{-1} of N per year with a nutrient balance of 16-8-12 [2 MgO, 12 SO_3, 2.6 CaO]). A seasonal growth monitoring of height and diameter was carried out along with dry biomass production and assessment of soil properties before and after of the trial was carried out. Irrigation and fertilisation significantly increased aboveground biomass production, averaging 20.6–55.4 t ha^{-1} per year; the combined treatments 0 mm – 0 kg ha^{-1} of N and 1298 mm–300 kg ha^{-1} of N were the least and the most productive, respectively. The data constitute a useful resource for the adjustment of the optimal irrigation (≥ 1500 mm per year of rainfall plus irrigation) and fertilisation doses (≥ 150 kg ha^{-1} of N) applied to plantations, as well as the management of crops to design a sustainable productive system that allows the preservation or improvement of soils. The energy and physical-mechanical biomass properties together with the derived pellets were of high quality, and they show promise for industrial boiler use.

1. Introduction

Eucalyptus spp. are the main source of lignocellulosic biomass used by commercial plantations, which equates to roughly 20 million ha worldwide [1] because of their rapid adaptability to different climatic conditions and easy use in plant breeding programs. The main biomass end products are cellulose pulp and the production of energy [2–4], as well as other finished products such as timber, furniture, etc. [5]. Plantations are mainly located in temperate areas, with exposure to mild winters and rainfall distributed throughout the year; however, they are also found in climates with a dry season, such as extended areas of the Iberian and Italian Peninsulas, Chile, South Africa and Australia [1].

The international commitments signed by most of developed States enforced the promotion of clean and renewable energy sources aiming to mitigate climate change effects [6,7], and biomass was among the energy sources. The worldwide energy production through renewable sources currently accounts for almost 20% of the global energy output, and biomass contributes up to 63% of the renewable sources [8]. Lignocellulosic biomass usage, although historically consolidated, is steadily expanding. To ensure supply for the growing demand without altering current agroforestry systems, traditional natural biomass exploitations need to be complemented with plantations of fast-growing tree species [9–11] for efficient land management. In addition, the development of lignocellulosic biomass production plantations in rural areas would aid local economies and maintain population levels while reducing CO_2 emissions [9,12,13].

In practice, lignocellulosic energy crops occupy degraded soils with low fertility. Nevertheless, they are required to provide a large amount of biomass in a cost-effective manner. In the case of eucalyptus trees, the main growth and survival limitations of the plantations tend to be water stress, lack of soil fertility and the winter frosts [14–16]. The loss of stem diameter growth during the dry season in Mediterranean climate regions makes irrigation necessary in order to achieve maximum

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production potential [16]. In the same manner, low soil fertility levels in plantations require the addition of mineral nutrients to achieve the desired production goals as well as to increase water and nutrient use efficiency [17–19]. Consequently, it is necessary to establish appropriate irrigation schedules that allow for a balance between the productivity maintenance and water use.

Accordingly, environmental diversity together with the need to improve production promotes the development of selection and breeding programmes capable of generating new taxa (species, clones, hybrids); it also promotes forestry improvement programmes and the assessment of adaptive capacity and productive potential [4,20,21]. The most widely used eucalyptus species in forest plantations and breeding programs are *Eucalyptus grandis*, *Eucalyptus globulus*, *Eucalyptus urophylla* and *Eucalyptus camaldulensis* [22], being *Eucalyptus x urograndis* one of the most important interspecific hybrids because it combines the rapid growth of *E. grandis* and the disease/climate tolerance of *E. urophylla* [23]. As far as we know, *E. x urograndis* has not been used in the Iberian Peninsula in commercial plantations, but other eucalypts. Commercial eucalyptus plantations in the region usually produce $3-25\,\mathrm{t\,ha^{-1}}$ per year of dry woody biomass with a fertilisation range of 0–100 $\mathrm{kg\,ha^{-1}}$ of N per year [24–26]. The species principally used in this region are *E. globulus*, *E. nitens* and *E. camaldulensis*, depending on the site characteristics. Apart from commercial plantations, unpublished field trials carried out with *E. globulus*, *E. camaldulensis*. *E. x trabutti*, *E. dunnii*, *E. maidenii* and *E x urograndis*, in which the authors of this article have participated, the resulting woody biomass production was 15–40 $\mathrm{t\,ha^{-1}}$ per year when annual fertilisation was applied up to 150 $\mathrm{kg\,ha^{-1}}$ of N. In the case of highly productive eucalypts such as *E. grandis* and *E. x urograndis* in fertilised commercial plantations in Brazil and Florida, $20-40\,\mathrm{t\,ha^{-1}}$ per year of woody biomass were obtained [26–28].

The forecast estimates for the year 2050 for lignocellulosic energy crops may represent approximately 5%–10% of the global forestry area. This makes it necessary to study the possible environmental effects [12,29] on soil fertility and productivity in areas with existing poor soil characteristics [30,31]. Due to the high economic and energy costs of mineral fertilisation, and unknown environmental effects of implementation that have yet to be precisely ascertained [32], it is necessary to establish and adjust the applications.

Therefore, the main objective of this study was to assess the biomass production for energy use of a clone of *Eucalyptus x urograndis* (hybrid between *E. grandis* and *E. urophylla*) under different water and nutritional availability regimes in a Mediterranean environment, under a short rotation coppice (three years), in addition to biomass property analysis. *E. x urograndis* usually produces high biomass yields at the cost of a high moisture and mineral nutrition demand, therefore plantations in poor soils and in environments with a dry period should be evaluated.

2. Material and methods

2.1. Plant material and experimental design

One-year-old *Eucalyptus x urograndis* nursery plants, provided by the Spanish pulp company ENCE, energía y celulosa S.A., were planted in a field trial located in Huelva (SW Europe, 37° 19′ 48.5″ N, 7° 18′ 51.8″ W, at 125 m). The vegetative material consisted of ramets belonging to the hybrid clone n° 5, $25-30$ cm height with a stem diameter of 3.5–5.0 mm, derived from rooted softwood cuttings. The plants were potted in 150 $\mathrm{cm^3}$ forest containers filled with coconut fibre, well-watered and fertilised and grown in an outdoor nursery for eight months before the planting date.

The experimental plot was located in a Mediterranean climate with mild winters and a marked summer period of 3–4 months. Mean temperature and annual rainfall in the area for the previous 20 years were 16 °C and 540 mm, respectively. The experimental plot was located in

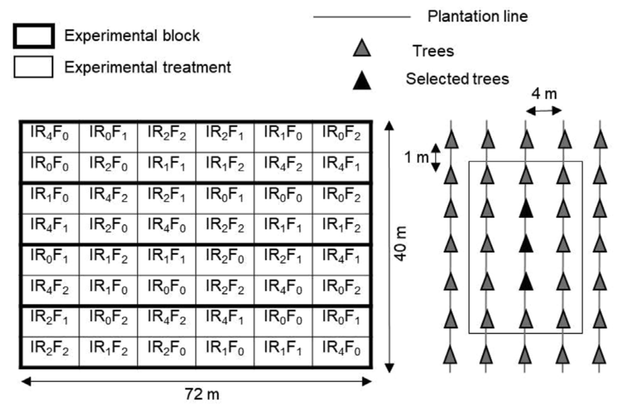

Fig. 1. Schematic representation of the experimental design. `$IR_x$´ are the irrigation treatments and `F_x´ the fertilisation treatments. Irrigation treatments (IR_0, IR_1, IR_2 and IR_4) corresponded to (0, 325, 646 and1298) mm of water per year, respectively; while fertilisation treatments (F_0, F_1 and F_2) corresponded, respectively, to (0, 150 and 300) kg $\mathrm{ha^{-1}}$ of N per year.

an abandoned citrus crop field. Soil texture was a sandy-loam type, with the top 20 cm having the following properties just before planting [mean (SE)]: pH = 4.8 (0.2); organic matter content, OM = 7.6 (0.4) g $\mathrm{kg^{-1}}$; electric conductivity, EC = 5.82 (0.73) mS $\mathrm{m^{-1}}$; bulk density, BD = 1.45 (0.03) kg $\mathrm{dm^{-3}}$; N content [Kjeldahl] = 0.32 (0.05) g $\mathrm{kg^{-1}}$, P content [Olsen] = 2.35 (0.08) mg $\mathrm{kg^{-1}}$, and exchangeable K = 45.7 (3.7) mg $\mathrm{kg^{-1}}$. Site preparation consisted of a linear subsoiling followed by a shallow tillage. A contact herbicide (oxyfluorfen, 24% weight to volume ratio) was applied before planting (1.5 $\mathrm{L\,ha^{-1}}$) and four months after planting (1.5 $\mathrm{L\,ha^{-1}}$). Plants were planted in mid-April 2011 in lines with a separation of 1 m between plants and 4 m between lines (a crop density of 2500 plants/ha). Plants were drip irrigated daily until the end of September 2011 to avoid mortality due to the typical drought period. During this period plants received a total of 325 mm of water and were fertilised (fertigation) with a nutrient ratio of 15-15-15 at a rate of 75 $\mathrm{kg\,ha^{-1}}$ of N.

The year thereafter, in June 2012, 4 experimental blocks with 180 trees each within the study plot were established (Fig. 1). At this date plants were 4.6 (0.5) m in height and the stem diameter at 5 cm above ground level was 63.4 (8.0) mm. A total of 12 cultivation treatments were randomly distributed within each block (Fig. 1) following a factorial design with 4 levels of irrigation (IR_0, IR_1, IR_2 and IR_4) corresponding to (0, 325, 646 and 1298) mm per year, and 3 levels of fertilisation (F_0, F_1 and F_2) corresponding to (0, 150 and 300) kg $\mathrm{ha^{-1}}$ of N per year, respectively. The basic experimental unit (cultivation treatment within each block) consisted of 3 rows of 5 trees (Fig. 1). Fertilisation was dissolved in the irrigation water using a 16-8-12 (2 MgO, 12 SO_3, 2.6 CaO) nutrient ratio. Micronutrients were also applied. In the cultivation treatments with no irrigation (IR_0F_x), fertilisation was applied twice a year (June and February) using a controlled release fertiliser [Basacote® Plus 6M 16-8-12 (2-10), containing 2% MgO, 10% of soluble SO_3, 12% total SO_3, and micronutrients] together with a 2.6 rate of CaO. Fertigation was applied from April to September during each year of the study. The precipitation regimes during the study period were 510 mm (June 2012 to May 2013) and 326 mm (June 2013 to May 2014), with the summer having the driest period [5.0 (4.3) % of rainfall)] (Fig. 2).

2.2. Growth and biomass assessment

Stem diameter (*D*, 5 cm above ground level) and plant height (*H*) measurements were taken on a total of 144 trees (3 trees per cultivation treatment and block) across 7 different dates. Selected trees were located in the central part of each experimental unit (Fig. 1) in order to avoid any edge effects. Stem diameter increment (SDI, mm per day) was

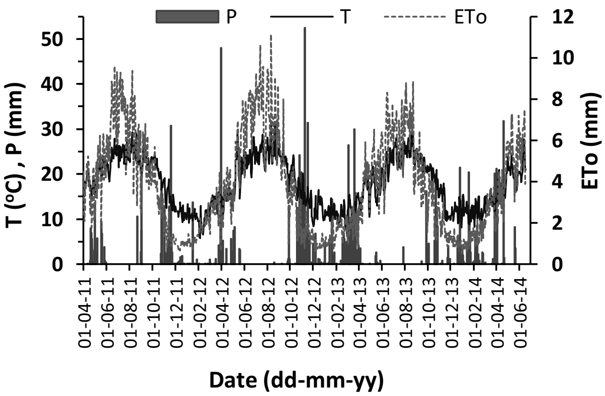

Fig. 2. Annual evolution of the mean (± SE) daily temperature (T) and the reference evapotranspiration (ETo) together with the trial plot precipitation (P).

used to assess the overall plant growth since the initial time of planting, calculated as the diameter difference between consecutive measurements, divided by the elapsed days.

During the entire study period, a total of 15 trees with stem diameters between 23 mm and 150 mm were used to evaluate the stem diameter and aboveground biomass relationship; the trees were randomly selected throughout the entire trial and belonging to all culture treatments, but were not the same trees that were used for growth assessment. Biomass was separated into three parts: thick stems and branches (with a cross section diameter > 25 mm, bark included), thin branches (diameter < 25 mm), and leaves. Samples were cleaned and oven-dried at 80 °C until attaining a constant weight. Since there were no significant differences between cultivation treatments in the allometric relationships of the trees of this field trial, aboveground biomass at the end of the cultivation period was estimated based on only one allometric relationship resulting from fitting the following equation to data ($X = \alpha D^{\beta}$) [14,18], constants being α and β, D the stem diameter (mm), and X the dry weight (g) of the total aboveground tree biomass (AGTB) or the leaves (LW): AGTB = 0.1243 $D^{2.5295}$ ($R^2 = 0.974$, $p < 0.001$); LW = 1.218 $D^{1.6463}$ ($R^2 = 0.944$, $p < 0.001$). AGTB comprised leaves, stems, branches, and bark. The thick woody fraction was approximately 85% of the aboveground woody biomass, while the LW to AGTB ratio varied from 0.50 for $D = 30$ mm, 0.26 for $D = 60$ mm to 0.12 for $D = 150$ mm, similar to the results previously obtained by Bouvet et al. [4] for hybrid eucalyptus plantations.

2.3. Soil and plant samples along with biomass properties

At the end of the study period (June 2014), soil, litterfall and plant samples were collected to carry out chemical analyses, one sample per block and treatment. Every soil sample was a mixture of four subsamples randomly taken close to the midpoint between two planting lines at both sides of the three trees selected and collected at a depth of 0 cm–20 cm. Soil samples were air dried and sieved (2 mm) before chemical analysis by standardized methods: pH (soil to distilled water ratio, 1:2.5); EC (5 g of dried soil in 25 cm^3 of distilled water); total organic carbon (TOC) and oxidizable organic matter fraction (OM) [Walkley and Black method]; total Nitrogen [Kjeldhal], available P [Olsen], available K, Ca and Mg [extracted with ammonium acetate and using an auto analyser Bran + Luebbe®, Model AIII]. Litterfall was sampled at the same soil sampling points, obtained from a 0.25 m^2 square area. Plant samples comprised three separated parts (leaves, thin branches and stems + thick branches) that were collected by mixing three subsamples taken from the middle part of the tree canopy of the three selected trees. Litterfall and plant samples were oven dried at 80 °C, weighted and stored in darkness at room temperature (15 − 20 °C) in sealed containers for subsequent analysis. Litterfall and plant materials were ground, passed through a 0.5 mm stainless-steel sieve, and analysed by standardized methods: N (Kjedahl), C (using an elemental analyser, Thermo Scientific™ FLASH 2000), Ca, P, K, S, Mg and micronutrients (ICP-OES, Thermo Jarrell Ash Corporation, after extraction with HNO_3). High and low heating values (constant volume) of leaves and wood were determined according to the UNE-EN 14918:2011 standard by using an automatic isoperibol calorimeter (Parr 6300®) and referred to a dry basis (moisture-free). Moisture content was measured by applying the standard ISO 18134-3:2015 (oven dried at 105 °C), and ash content was measured by applying the standard ISO 18122:2015 (550 °C). Pellets were manufactured using a pelleting press (PLT-400, Smartec®, Italy). Plant materials were milled (Woodstock 3PH, Smartec®, Italy) and sieved to a particle size of 0.2 mm–5.0 mm in order to create homogenous samples. The sample (i.e., sawdust) moisture content was set to 120 g kg^{-1} with a bulk density of 205 kg m^{-3} and an operating temperature ranging between 95 °C to 105 °C. The die channels had a diameter of 6 mm; the first part had a cone-shaped opening 3.5 mm deep and 70° angles; the active part was 22 mm long; the compression ratio was 3.67. Previously, die channels with a length of 20 mm–28 mm as well as sawdust subsamples set to a moisture content of 70 g kg^{-1} to 170 g kg^{-1} had been tested in order to choose the best possible option. Afterwards, the mechanical durability, moisture content, length and diameter, and the bulk density of the pellets were determined according to the ISO 17225-2:2014 standard.

2.4. Data analysis

The SDI was evaluated in the same trees during 6 different periods, so our data structure resulted in repeated measurements for each tree. Hence, as the within-tree observations were autocorrelated, we proceeded to use a linear mixed model for which the tree (within block) was considered a random effect. Irrigation (*IR*), fertilisation (*F*) and the interaction (*IR x F*) were included as fixed effects. The growth seasonality was evaluated by introducing the growth evaluation period (date) as a fixed effect. We also assessed block growth differences. Thus, our full model (equation 1) had the following structure:

$$SDI = Block + Date + IR + F + IR \times F$$

The best model was selected by using a backward stepwise procedure based on the corrected Akaike's Information Criterion (AICc) [33]. The full model (equation (1)) was compared with models lacking one of the fixed effects, so the relative importance of each effect could be assessed by comparing the AICc reduction after removal. For those fixed effects retained in the final model, the factor level differences (e.g., between irrigation treatments) were evaluated by the least-squares method followed by a Tukey HSD test.

The fertigation effect on the aboveground tree biomass production was assessed by using a linear model in which *IR*, *F* and *IR x F* were included as fixed effects. We also included the block as a fixed effect, and accounted for by the effect of the initial tree size by including the stem diameter measurement at the beginning of the fertigation experiment (D_o) as a covariate. The full model (2) applied was:

$$AGTB = D_o + Block + IR + F + IR \times F$$

The model selection was also performed based on the AICc, differences between factor levels were evaluated by a Tukey HSD test. For the soil, litterfall and plant samples, an analysis of variance was carried out in order to determine the statistically significant differences between treatments, including *Block*, *IR*, *F* and *IR x F* as fixed effects. Significant differences were established at $\alpha = 0.05$. To evaluate the among-treatment comparisons, the Tukey HSD or T3-Dunnett tests were used in order to differentiate within of the homogeneous groups (according to the variance homoscedasticity). All statistical analyses were performed in R using version 3.2.3, packages *lme4* and *stats* were used to fit the mixed and linear models, respectively. Contrasts between factor levels were performed with the package *lsmeans*.

Table 1
Model comparison for tree growth (Stem Diameter Increment (SDI) model) and aboveground tree biomass (Biomass model) using the corrected Akaike's information criterion (AICc). The 'No block', 'No Date', 'No D_0', 'No Irrigation', 'No Fertilisation', and 'No interaction' models did not include the effect of the block, measurement date, initial tree trunk diameter, irrigation, fertilisation, and their interactions, respectively. ΔAICc is the difference in AICc between the evaluated model and the full model. The null model ignored all evaluated terms (i.e., intercept-only model). The best fitting model (selected model) included the Date, Irrigation and Fertilisation for SDI, and D_0, Irrigation and Fertilisation for Biomass.

Model	SDI model		Biomass model	
	AICc	ΔAICc	AICc	ΔAICc
full	2894.0		2985.7	
no Block	2892.6	−1.4	2977.8	−7.9
no Date	3293.6	399.6	–	–
no D_0	–	–	3040.3	54.6
no Irrigation	2925.6	31.6	3022.8	37.1
no Fertilisation	2919.3	25.3	2999.8	14.1
no Interaction	2888.2	−5.8	2981.2	−4.5
null	3324.2		3111.8	
Selected model	2812.9		2977.8	

3. Results

3.1. Plant growth and biomass production

No plant mortality occurred during the study period. However, during the third year, 3% of the trees suffered from stem breakage or leaning caused by wind and were not included in the measurements taken. The diameter increase in the two study years (from June 2012 to June 2014) varied from 55.7 (5.4) mm of IR_0F_0 to 99.8 (4.7) mm of IR_4F_2. All treatments considered, average heights achieved by the trees were 8.8 (0,8) m and 12.0 (1.1) m in June 2013 and June 2014, respectively.

The best SDI model included the growth evaluation date together with the irrigation and fertilisation effects (Table 1). As expected, the time component (i.e., date) was the most important factor as indicated by a larger AICc increase when removed from the model (Table 1). Both, irrigation and fertilisation effects were retained in the final model, although the AICc increase was larger when the irrigation term was dropped from the model. Similarly, irrigation and fertilisation terms, as well as D_o, were also retained in the final model explaining the tree biomass, while the block and the *IR x F* interaction were not included in any model as indicated by the model's improvement (i.e., lower AICc) following the removal of these terms (Table 1).

Stem diameters grew at high rates during the entire experiment 0.109 (0.022) mm day^{-1}, with the greatest SDI evidenced the first autumn following the application of the fertigation treatments (Fig. 3). Both, SDI and AGTB increased with higher irrigation and fertilisation rates (Figs. 4 and 5). No stem diameter increase was observed during the dry season (June–September) in the treatments lacking irrigation (IR_0F_x). In terms of total dry biomass production (AGTB), the fertigation experiment averaged 40.96 (14.74) t ha^{-1} per year, with the highest value from the IR_4F_2 treatment (55.40 t ha^{-1} per year), which was approximately 169% higher than the IR_0F_0 treatment (20.60 t ha^{-1} per year). Compared with the IR_0 treatment, IR_1, IR_2 and IR_4 increased biomass production by 11.8%, 16.9% and 36.7%, respectively, while compared with the F_0 treatment, F_1 and F_2 increased biomass production by 22.4% and 54.6%, respectively.

3.2. Soil horizon effects and biomass properties

At the end of the trial, the irrigation (*IR*) effect, the *IR x F* interaction or the block proved not to be significant either for the physico-chemical properties measured in the most superficial soil layer (0 − 20 cm), or in the leaf litter or in the aboveground biomass ($0.056 < p < 0.965$).

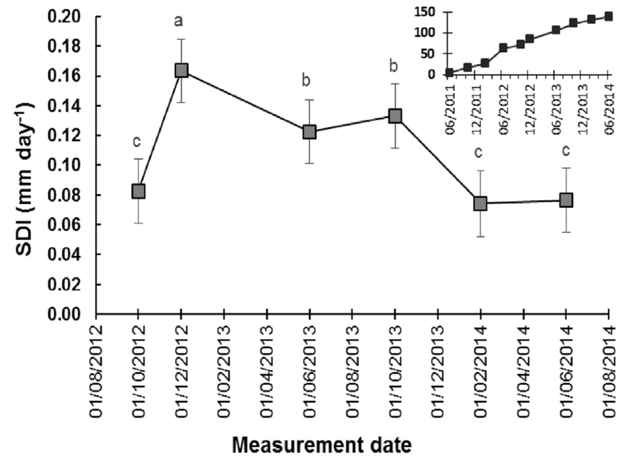

Fig. 3. Stem diameter increment (mean ± SD) during the period in which fertigation treatments were applied. Different letters indicate significant differences between the evaluation periods. Figure in the right corner shows the diameter evolution (mm) from the moment of plantation (15 April 2011) until the end of the study (12 June 2014). Application of fertigation treatments begun in June 2012.

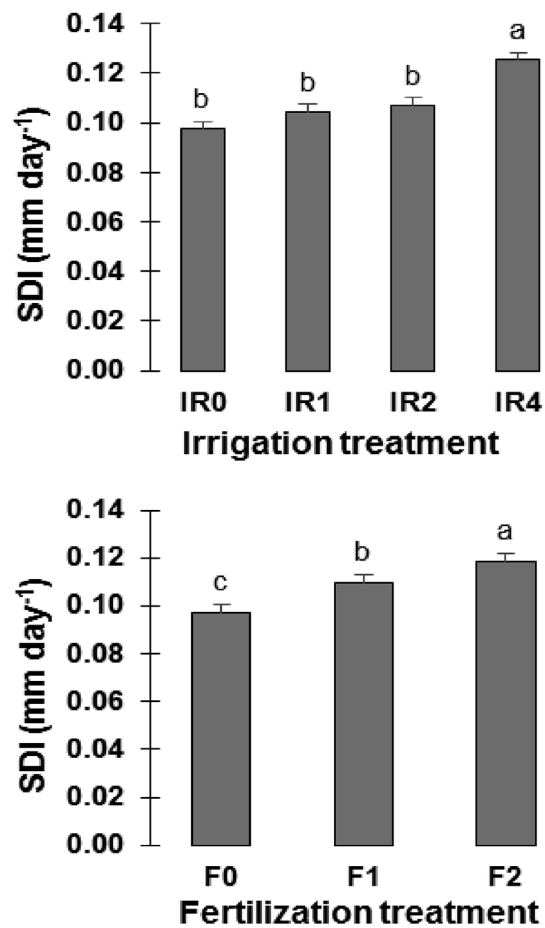

Fig. 4. Stem diameter increment (mean ± SE) for the different irrigation (IR_0 to IR_4) and fertilisation (F_0 to F_2) treatments. Different letters depict significant differences between the irrigation and fertilisation treatments.

The fertilisation effect was also not significant ($0.139 < p < 0.927$) for most of the parameters analysed (Table 2), with the exception of the soil pH, P and K contents, along with the litterfall dry weight (Table 3).

During the time course of this study, no important changes were observed regarding the superficial soil horizon properties (see subsection 2.1, and Tables 2 and 3). Consequently, considering the mean soil

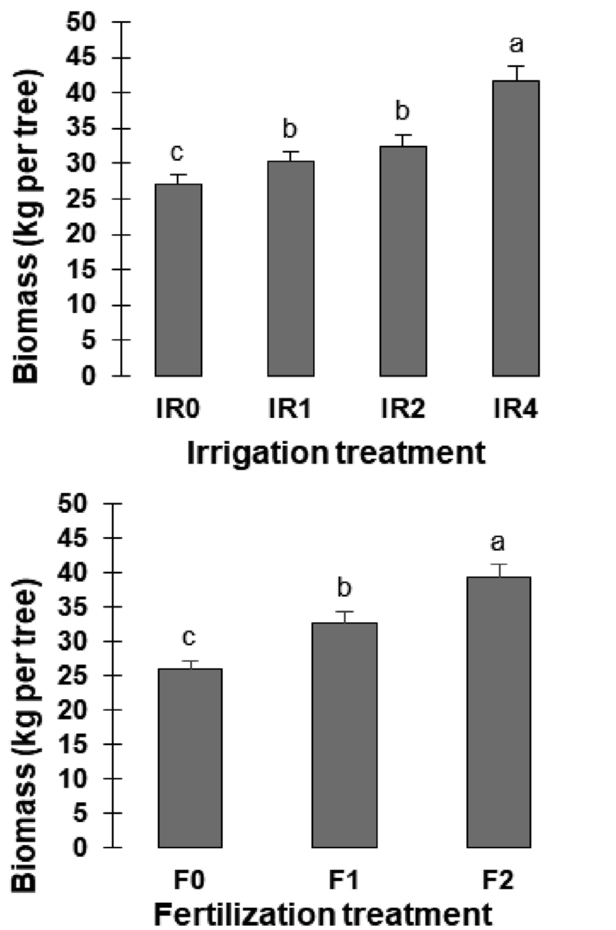

Fig. 5. Aboveground tree biomass (mean ± SE) for the different irrigation (IR_0 to IR_4) and fertilisation (F_0 to F_2) treatments. Different letters reveal significant differences between the irrigation and fertilisation treatments.

property values and those of biomass production, a preliminary estimate was drawn involving the N, P and K nutrients, the soil variation along of the three years, together with the leaf litter reserves and the aboveground biomass (Table 4).

The *E. x urograndis* pellet characteristics are displayed in Table 5, which also displays other pellets made out of other commonly used tree species for energy purposes, including well established crops (*Eucalyptus camaldulensis*, *Populus x euroamericana*) and natural forests (*Pinus pinea*) as a comparison. Taking into account only the thick woody biomass fraction (stem + thick branches), the biomass production

Table 3
Observed pH, P [Olsen] and exchangeable K contents of the soil layer (0–20 cm), and the residual soil surface litterfall at the end of the study period [mean (SE)]. *p*: significance level between fertilisation treatments. Different letters in the same column indicate significant differences.

	Soil layer			Litterfall
	pH	P (mg kg^{-1})	K (mg kg^{-1})	dry weight (g m^{-2})
F_0	4.99 (0.06) b	1.01 (0.09) a	17.8 (1.7) a	580 (52) a
F_1	4.67 (0.08) a	1.47 (0.19) ab	29.6 (3.6) b	723 (65) b
F_2	4.75 (0.11) ab	2.71 (0.78) b	35.6 (5.6) b	700 (38) b
p	0.026	0.038	0.003	0.042

Table 4
Difference between soil N, P and K contents before and after of the cultivation period (April-2011 to June-2014), and nutrient contents contained in the litterfall and the aboveground biomass at the end of the study period. Mean values for all of the irrigation and fertilisation treatments as a whole are shown.[a]

	Soil layer (0–20 cm)	Litterfall	Leaves	Thin branches	Stem + thick branches
N (kg ha^{-1})	−290.0[b]	50.08	259.4	30.1	173.7
P (kg ha^{-1})	−2.03[b]	2.67	15.07	4.63	20.85
K (kg ha^{-1})	−52.2[b]	9.4	92.6	38.6	132.0

[a] Mean values: tree diameter at the end of the study period, D = 143 mm; soil bulk density = 1.45 kg dm^{-3}; tree density 2500 trees ha^{-1}; AGTB = 0.1243 $D^{2.5295}$ = 35,187.92 g per tree (~87 969.8 kg ha^{-1}); leaf biomass, LW = 1.218 $D^{1.6463}$ = 4305.04 g per tree (~10 762.6 kg ha^{-1}); thin branches biomass = 0.1 (AGTB – LW) = 3088.29 g per tree (~7720.7 kg ha^{-1}); soil layer volume = 0.2 m x 10 000 m^2 = 2000 m^3 (~2900 t); litterfall = 6676.67 kg ha^{-1}.
[b] Soil nutrient content variations from the beginning to the end of the study period (see subsection 2.1 and Table 2).

(subsection 3.1.), and the heating value of the biomass (Table 5), the energy yield of the different treatments averaged 340 – 913 MJ ha^{-1} per year, in terms of HHV, and 294 – 789 MJ ha^{-1} per year in terms of LHV.

3.3. Production costs

By considering the production costs and the income derived from the sale of thick woody biomass (85% of AGTB at the time of harvest) a brief economic balance is shown on Table 6. Thin branches and leaves have not been included because of their low quality for energy purposes [37,38]. Compared with the IR_0 treatment, IR_1 increased unit costs of biomass and energy production (i.e., € t^{-1} and € GJ^{-1}) by 3.0%, but IR_2 and IR_4 respectively decreased these costs by 2.1% and 21.1%; while compared with the F_0 treatment, F_1 and F_2 increased theme costs

Table 2
Soil, litterfall and aboveground biomass properties for all treatments as a whole at the end of the study period [mean (SE)]. *na*: not analysed.

	Soil layer (0–20 cm)	Litterfall	Leaves	Thin branches	Stem + thick branches
N (g kg^{-1})	0.22 (0.07)	7.5 (0.7)	24.1 (1.2)	3.9 (0.7)	2.5 (0.6)
P (g kg^{-1})	0.002 (0.001)[a]	0.4 (0.1)	1.4 (0.3)	0.6 (0.1)	0.3 (0.1)
K (g kg^{-1})	0.027 (0.004)[a]	1.4 (0.1)	8.6 (0.7)	5.0 (0.6)	1.9 (0.7)
Ca (g kg^{-1})	0.09 (0.02)	14.6 (0.8)	15.9 (1.3)	15.7 (1.5)	8.5 (1.2)
Mg (g kg^{-1})	0.017 (0.001)	2.7 (0.1)	2.6 (0.3)	1.3 (0.2)	1.5 (0.3)
S (g kg^{-1})	*na*	0.7 (0.1)	1.6 (0.3)	0.3 (0.1)	0.2 (0.1)
Cl (g kg^{-1})	*na*	0.28 (0.09)	1.70 (0.07)	0.88 (0.03)	1.05 (0.04)
B (mg kg^{-1})	*na*	38.3 (1.7)	68.4 (3.2)	10.7 (1.4)	8.4 (1.2)
Fe (mg kg^{-1})	*na*	252 (29)	397 (32)	67 (15)	30 (13)
Mn (mg kg^{-1})	*na*	381 (27)	37 (5)	10 (3)	12 (4)
C (g kg^{-1})	4.2 (0.3)[b]	495 (12)	469 (10)	444 (10)	441 (9)
OM (g kg^{-1})	7.2 (0.4)	*na*	*na*	*na*	*na*
EC (mS m^{-1})	5.6 (0.4)	*na*	*na*	*na*	*na*

[a] Significant differences between fertilisation treatments displayed in Table 3.
[b] For the soil layer the total organic carbon (TOC) is presented.

Table 5
Physico-mechanical and chemical properties [mean (SE)]of the pellets made out of *E. x urograndis* as well as other species grown at the same site and during the same period (*E. camaldulensis* and *Populus x euroamericana* 'I-214'), or from 15-year-old trees growing in a forest stand 100 m away from the study plot (*Pinus pinea*). *L*: pellet length; *Dp*: pellet diameter; MD: mechanical durability; HHV, LHV: high and low heating value, respectively; BD: bulk density; PD: particle or pellet density; PEF: pellet efficiency (i.e., pellet to sawdust dry weight ratio after pelletization); Bark: bark to wood dry weight ratio. ChL: the length of the die channel used for making the pellets.

	Eucalyptus x urograndis		*Eucalyptus camaldulensis*	*Populus x 'I-214'*	*Pinus pinea*[a]
	Stem + thick branches	Thin branches	Stem + thick branches	Stem + thick branches	Stem + thick branches
L (mm)[b]	21.5 (8.1)	21.5 (7.5)	22.2 (6.8)	22.3 (7.2)	23.2 (6.9)
Dp (mm) [b]	6.01 (0.02)	6.07 (0.02)	5.99 (0.02)	6.02 (0.01)	6.01 (0.01)
Moisture (%)[b,c]	6.4 (0.5)	7.0 (0.6)	6.5 (0.6)	6.8 (0.6)	7.2 (0.5)
Ash (%)[c]	1.2 (0.3)	3.2 (0.4)	2.2 (0.3)	1.0 (0.3)	0.6 (0.3)
MD (%)[b]	96.5 (3.0)	97.2 (3.5)	94.3 (2.9)	96.1 (2.5)	98.3 (1.7)
HHV(MJ kg^{-1})[d]	19.40 (0.36)	19.37 (0.32)	18.54 (0.29)	19.44 (0.29)	20.41 (0.32)
LHV(MJ kg^{-1})[e]	16.74 (0.29)	16.16 (0.25)	15.91 (0.22)	16.70 (0.25)	17.50 (0.25)
BD (kg m^{-3})[b,e]	694 (10)	630 (15)	675 (12)	690 (16)	635 (15)
PD (kg m^{-3})[e]	1330 (50)	1250 (65)	1266 (28)	1310 (25)	1201 (32)
PEF (%)	98.8 (2.0)	99.2 (2.1)	98.9 (2.0)	99.2 (1.7)	99.5 (0.8)
Bark (%)	16.3 (2.0)	21.1 (1.8)	15.9 (2.5)	15.4 (1.8)	22 (2.7)
ChL (mm)	22	22	20	24	28

[a] The bark was included in the pelletization process except for *P. pinea*.
[b] According to ISO 17225-2:2014.
[c] Mass fraction.
[d] Referred to a dry basis (moisture-free, after oven-drying at 105 °C).
[e] Referred to a wet basis (the moisture content of pellets as received, i.e. the moisture shown above).

Table 6
Mean values of accounting costs of biomass production, dry thick woody biomass produced, gross income from the sale of wood, profit (income minus costs), and unit costs of energy production when energy is calculated as HHV dry basis. Irrigation (IR_x) and fertilisation (F_x) effects, as well as the two more extreme combined treatments (IR_0F_0, IR_4F_2), are shown.

	Accounting cost (C) (€ ha^{-1} $year^{-1}$)	Woody biomass (t ha^{-1} $year^{-1}$)	Gross income (I) (€ ha^{-1} $year^{-1}$)	Balance (I − C) (€ ha^{-1} $year^{-1}$)	Costs of energy (€ GJ^{-1})
Irrigation effect					
IR_0	1517.2	24.2	1695.8	178.6	3.23
IR_1	1748.8	27.1	1898.1	149.3	3.32
IR_2	1786.8	29.2	2040.8	254.0	3.16
IR_4	1879.5	38.3	2677.5	798.0	2.53
Fertilisation effect					
F_0	1183.9	23.2	1624.4	440.5	2.63
F_1	1719.7	29.9	2094.4	374.8	2.96
F_2	2253.1	35.9	2510.9	257.8	3.24
Combined treatments					
IR_0F_0	981.4	17.5	1225.7	244.3	2.89
IR_4F_2	2421.7	47.1	3296.3	874.7	2.65

Calculations consider a plantation rotation of 15 years with a harvest every 3 years (first year plantation set up and 14 years of production with 5 harvests). The rates and wood price have been obtained from the companies TRAGSA [34] and ENCE [35], the two largest Spanish forestry companies. The price of dry wood has been set at 70 € t^{-1}. Among the range of possible costs for these forestry works, intermediate ones have been considered. It has also been taken into account the calculation methodology used by Wit and Faaij [36]. The fixed costs considered were: site preparation (subsoilingg and tilling), 22.9 € ha^{-1} per year; plants and planting, 121 € ha^{-1} per year; harvesting processing and piling, 625 € ha^{-1} per year; irrigation (materials, installation and supply rates), 190.47 € ha^{-1} per year; other fixed costs (land, herbicide, etc.), 160 € ha^{-1} per year. Whereas the variable costs were: loading and transport by truck 3 € t^{-1}; irrigation (water and energy), 0.1 € m^{-3}; fertiliser, 0.55 € kg^{-1}. Financial costs and extra costs have not been taken into account.

by 12.7% and 23.1%, respectively. In the same way, in relation to the estimated profits (i.e., € ha^{-1} per year), compared with the IR_0 treatment, IR_1 decreased them by 16.4%, but IR_2 and IR_4 respectively increased them by 42.3% and 346.8%; whereas compared with the F_0 treatment, F_1 and F_2 decreased profits by 14.9% and 41.5%, respectively.

4. Discussion

4.1. Plant growth and biomass production

The results showed a high *E. x urograndis* growth rate maintained throughout the year, which was increased by fertilisation and irrigation. Diameter growth increased in fall and spring without a winter decline nor a summer stop in the irrigated treatments. However, the reduced growth rate during the driest spring (2014) and the complete growth arrest during the summer in the non-irrigated treatments highlight this species limited drought resistance. The surprising size reached by the trees (i.e., *D*, *H*) and the produced biomass are characteristic of the maximum growth exhibited by this species under favourable conditions (about 40 t ha^{-1} per year) [18,39–41] as well as that of other related species (e.g., *E. grandis*) [42]. It should be noted that in this study we analysed the total aboveground dry biomass, which includes leaves, branches and the trunk with bark. However, in the reduced water availability (IR_0F_x) treatments, production was also high, equalling or surpassing other *Eucalyptus* species (3 − 25 t ha^{-1} per year of dry woody biomass) [25,26,42] as well as other woody crops grown in a Mediterranean climate (3 − 23 t ha^{-1} per year) [13,43,44]; it should be remarked that this study was conducted on land that received an annual average of 418 mm rainfall. Nevertheless, caution is needed to extrapolate these results to commercial plantings, since the study plot dimensions allowed for a high soil homogeneity and cultivation, as well as a high plant survival.

Both irrigation and fertilisation contributions independently improved growth and biomass production. This relative irrigation and fertilisation independence could be due to the differential effects they originate on evapotranspiration, basal and leaf area, leaf area index or photosynthesis [19,45]. The synergistic effect of simultaneously providing both factors may have increased the trees' productive potential when 1500 mm per year (irrigation + rainfall) was exceeded accompanied by 300 kg ha^{-1} of N (IR_4F_2), possibly due to a resource use efficiency increase under these conditions [17–19]. Despite this, in the study area with an average annual rainfall of 540 mm, economic and environmental factors would seem to limit the attempt to provide plantations with more than 1000 mm of water per year through irrigation and the necessary additional fertilisation in order to achieve an optimal production [17,32,46], so it would be advisable to implement plantations in high water resource available areas.

Therefore, if the results are analysed in economic terms, irrigation system was not profitable when the annual irrigation contribution was only 325 mm (i.e., IR_1 treatment, 743 mm of supplied water taken into account rainfall and irrigation), since the unit cost of biomass production (€ t^{-1}) increased and benefits decreased compared with the non-irrigation treatment. However, when the water supplied exceeded 1000 mm (IR_2) the yield improved thanks to the reduction of unit costs and the improvement of economic returns. Likewise, when the water supplied exceeded 1500 mm (IR_4) this hybrid showed its great growth potential for use in commercial plantations. On the other hand, unlike irrigation, fertilisation increased the unit cost of biomass production to a greater extent than growth, so the benefits were reduced, with especial intensity for the most fertilised treatment (300 kg ha^{-1} of N per year). Therefore, the amount of fertiliser should be adjusted to the minimum amount necessary to promote growth and replace the nutrients removed in the harvested biomass. For this study, up to 150 kg ha^{-1} of N per year could be supplied without an economic damage greater than 15% at the same time that mineral requirements were satisfied.

The surplus of agri-food products, the abandonment of farmlands, as well as the energy deficit mean that the future plans of the European Union involve increasing the area of land dedicated to energy crops [47]. In Europe there are up to 20×10^6 ha of marginal or degraded agricultural land where these crops could be implanted [48]. Among them, more than 2.5×10^6 ha are in the Iberian Peninsula and about 10% of them would be potentially appropriate for the establishment of *E. x urograndis* plantations, increasing this proportion if irrigation water is available [49,50]. According to the last two authors, the most widely implanted eucalyptus species in the region are *E. globulus* and *E. camaldulensis*, but in many areas they are being replaced by other eucalypts: due to problems related to pathologies and cold climate for the former (e.g. replaced by *E. nitens* in the north of Iberian Peninsula), while for the latter is due to ecological problems. In this context, and not expecting commercial yields greater than those obtained in this trial, *E. x urograndis* could be planted in new lands (degraded or abandoned by agriculture) or it could replace other established eucalypts, in order to take advantage of its high growth capacity and its potential of adaptation to different environments [23].

4.2. Soil horizon effects and biomass properties

The short trial period of three years did not appear to cause major soil composition changes. For example, not even in the most superficial soil horizon were the pH, electrical conductivity, or organic matter significantly affected with respect to the initial soil state. However, Tables 3 and 4 show possible changes in soil conditions in high production plantations in the medium and long term possibly due to the nutrient cycle between the soil, the leaf litter and the aboveground biomass [51,52], particularly if the last is removed by harvests. Despite the nutrients provided by fertilisation applied during the three years (on average 375 kg ha^{-1} of N, 98.1 kg ha^{-1} of P and 243.5 kg ha^{-1} of K), the mineral element content decreased in the soil, accumulating most in the aboveground plant parts, as Bouvet et al. [4] had also determined. Leaf litter also maintains a nutrient reservoir role by slowly releasing elements to the soil and plants, while enhancing the mull type humus, a soil characteristic of Eucalyptus plantations [51,53]. However, as the nutrient amount preserved in the leaf litter during this study is not enough to compensate for the biomass withdrawals, and in addition to the slow decomposition rate that would further diminish release [54], additional fertiliser contributions are required in order to compensate [4,52]. Further, farm management practices not expecting to utilize low quality tree biomass such as leaves, thin branches or bark and leave them on site, or return the residual combustion ashes, could aid to recycle nutrients and to achieve the sustainability of system [38,55,56].

The energy and chemical properties of the biomass, as well as the physico-mechanical properties of the manufactured pellets evidenced appropriateness for energy use according to the international standards [57], particularly the trunk and the thick branches, which can be made into chips and pellets. The resulting pellets possessed hardwood characteristics [43,58,59] and were of a better quality than herbaceous species pellets but did not reach the maximum standardized quality of pellets derived from conifer debarked wood (e.g., *Pinus pinea*). Nonetheless, the physico-mechanical properties of the pellets provide a quality for non-industrial use (e.g., EN-Plus B quality [57]), however the high Cl content relegates them to high quality pellets for industrial use. It would be desirable to study the feasibility of debarking the *Eucalyptus x urograndis* wood and to analyse the resulting product to determine its' effect on quality.

In conclusion, we emphasize that *Eucalyptus x urograndis* adapted well to the Mediterranean edaphic-climatic conditions in SW Europe. It requires about 1000 mm of water per year to equalise the biomass production of the best energy crops in the region, but when annual contribution of water exceeds 1500 mm and fertiliser 150 kg ha^{-1} de N it develops its true productive potential compared to other *Eucalyptus* species. It can be exploited as a short rotation coppice energy crop (3 years) owing to its rapid growth (> 20 t ha^{-1} per year of aboveground dry biomass) and high biomass quality. The crop does not affect soil, but could improve degraded soils if the crop is properly managed.

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Non-standard abbreviations

AGTB: dry weight of the total aboveground tree biomass (g)
BD: bulk density (kg m^{-3})
ChL: the length of the die channel used for making the pellets (mm)
D: stem diameter measured 5 cm above ground level (mm)
Dp: pellet diameter (mm)
H: plant height (m)

HHV: high heating value (MJ kg^{-1})
L: pellet length (mm)
LHV: low heating value (MJ kg^{-1})
LW: dry weight of leaves (g)
MD: mechanical durability (%)
OM: oxidizable organic matter fraction (g kg^{-1})
PD: particle or pellet density (kg m^{-3})
PEF: pellet efficiency (i.e. pellet to sawdust dry weight ratio after pelletization) (%)
SDI: stem diameter increment (mm per day)
TOC: Total organic carbon (g kg^{-1})